TGF-β3 Stimulates and Regulates Collagen Synthesis Through TGF-β1– Dependent and Independent Mechanisms

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In contrast to the TGF-β1 and β2 isoforms, TGF-β3 has shown the ability to downregulate scarring and fibrosis *in vivo* under certain experimental conditions. In this study, we determined the direct effects of TGF-β3 on cultures of human dermal fibroblasts. TGF-β3 (0.1 to 100 pg per ml) increased DNA synthesis up to 50% ($p < 0.01$, $r = 0.970$), collagen protein synthesis up to 200% (dose range of 0.1 to 5 ng per ml, $p < 0.001$, $r = 0.990$), and increased α1(I) procollagen mRNA levels ($r = 0.999$), with maximal effects (200% of control) observed by 24 h. Collagen lattice contraction was increased by more than 50% in response to TGF-β3 ($p < 0.001$), and to a similar extent as the TGF-β1 isoform. Stimulation of collagen synthesis and of α1(I) procollagen mRNA levels in response to TGF-β3 was partially blocked by a TGF-β1–specific anti-sense oligonucleotide but was still detectable (35% greater than baseline) when TGF-β3 was added to dermal fibroblasts from TGF-β1 knock-out mice. In contrast with these stimulatory effects, however, downregulation of α1(I) procollagen, α1(III) procollagen, and TGF-β1 mRNA levels toward baseline occurred when TGF-β3 (0.1 to 5 ng per ml) was added simultaneously and in combination with TGF-β1. We conclude that stimulation of collagen synthesis by TGF-β3 occurs through TGF-β1–dependent and independent pathways. By downregulating the response to TGF-β1 and by shifting from one pathway to the other, TGF-β3 can dampen and provide fine-tuning to the overall TGF-β's induced program of collagen deposition. ***Key words: fibroblasts/fibrosis/isoforms/scarring. J Invest Dermatol 108:258–262, 1997***

The transforming growth factor-β's (TGF-β's) are a family of multifunctional peptides that regulate cell growth and differentiation through the action of distinct and common cellular receptors (Roberts and Sporn, 1990, 1993). TGF-β's are important in developmental processes and in regulating the immune response and play a fundamental role in extracellular matrix formation (Roberts and Sporn, 1993). They have been shown to accelerate many of the parameters of tissue repair (Roberts, 1995), and their pathogenic role in the development of fibrosis is becoming increasingly clear (Border and Noble, 1994). TGF-β1 was purified and characterized in 1983 as a homodimeric, 25 kDa protein. More recently, TGF-β's 2 through 5 have been discovered and characterized at the protein and molecular level. Presently, only the TGF-β1, β2, and β3 isoforms have been isolated from human sources (Cox, 1995). All members of the TGF-β family are synthesized as a large precursor protein, which is proteolytically cleaved to yield a mature carboxy-terminal unit of 112 amino acids (Roberts, 1995). The TGF-β peptides have a high (70–80%) degree of shared sequence homology in the carboxy-terminal mature region, and the sequence of all three isoforms is almost totally conserved across species (Roberts, 1995). Interestingly, however, the *N*-terminal pro-domain of the TGF-β3 isoform is quite distinct from the other two mammalian isoforms, although highly conserved (80–90%) across species.

The TGF-β1, β2, and β3 isoforms are differentially regulated, but their different effects and interactions are not clear. Although it has often been stated that the three peptides are equivalent in most assays, differences have been noted in the localization and distribution of the three isoforms in several tissues during embryogenesis, fibrosis, and wound healing (Graycar *et al*, 1989; Roberts and Sporn, 1990). A recent report suggests a markedly different effect of TGF-β3 from the other isoforms. In that study, injection of TGF-β3 into experimental murine wounds decreased scarring, the opposite of what was invariably observed with injection of the β1 or β2 isoforms (Shah *et al*, 1995). It is unclear whether this paradoxical action is due to differential effects of TGF-β isoforms on the synthesis of extracellular matrix by fibroblasts. Therefore, we decided to investigate the direct effect of TGF-β3 and its interactions with TGF-β1 on collagen synthesis in human dermal fibroblasts. The results shown here indicate that TGF-β3 stimulates collagen synthesis through TGF-β1–dependent and independent mechanisms and that it is able to control and block the stimulatory effects of TGF-β1.

MATERIALS AND METHODS

Dermal Fibroblast Cultures Cultures of human dermal fibroblasts were established and maintained, as previously described, from foreskin of healthy neonatal infants (Falanga *et al*, 1991). Passages 3 to 8 were used throughout the experiments. Cultures were initiated from explants and passaged in T-75 flasks (Corning Glass Works, Corning, NY) and seeded in Dulbecco modified Eagle's medium (DMEM, from Sigma, St. Louis, MO)

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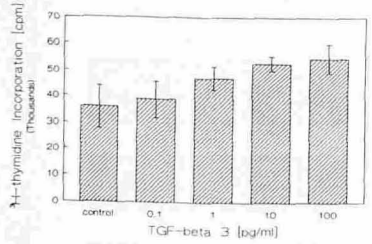

Figure 1. TGF-β3 stimulates DNA synthesis in human dermal fibroblasts. Cells were exposed to increasing concentrations of TGF-β3 for 24 h before measurements were made. Results represent the mean ± SD from quadruplicate wells.

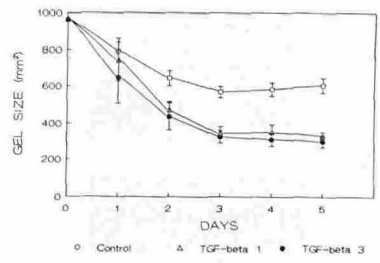

Figure 3. TGF-β3 stimulates collagen gel contraction. Gels consisting of type I collagen and human dermal fibroblasts were incubated in the presence of control media (MCDB 105) with or without TGF-β1 or TGF-β3. Gel diameter was measured daily. The results are the mean ± SD from quadruplicate wells.

and 10% fetal bovine serum (FBS) (GIBCO laboratories, Grand Island, NY). Fibroblasts were grown and kept at 37°C in 5% CO_2, 95% air. Experiments were done by washing monolayers with plain DMEM and incubating the cultures 30 min with 0.1% bovine serum albumin (Sigma). Serum-free medium (AIM-V, GIBCO BRL, Gaithersburg, MD) was used in experiments designed to determine differential effects of TGF-β isoforms. For the purpose of establishing cultures of TGF-β1 knock-out dermal fibroblasts, skin from TGF-β1 knock-out mice (Kulkarni *et al*, 1993) and control littermates was obtained from Dr. Anita Roberts (National Cancer Institute, Laboratory of Chemoprevention). Dermal fibroblasts cultured from mouse skin were maintained in DMEM plus 10% FBS until actual experiments, at which time cultures were washed extensively with DMEM, and serum-free medium (AIM-V) was added.

[^{3}H]Thymidine Uptake Fibroblasts were seeded at 30,000 cells per 2 cm^2 wells in DMEM supplemented with 10% FBS. After 24 h, monolayers were washed with phosphate buffered saline (GIBCO BRL) and the medium was replaced with 1 ml of DMEM and 0.5% HyClone serum (HyClone Laboratories, Logan, UT). After an additional 24 h, monolayers were washed with phosphate-buffered saline and incubated for 24 h with 1 ml of DMEM, 0.5% Hyclone serum, with, and without TGF-β's. Next day, cultures were each pulsed for 4 h with 2 μCi of methyl-[^{3}H]thymidine (86 Ci/mmol, Amersham Corp., Arlington Heights, IL). Measurements of [^{3}H]thymidine uptake were done as previously described (Takagi *et al*, 1995).

RNA Extraction and Northern Analysis Total cellular RNA from cells was isolated by extraction in guanidium isothiocyanate, using the method of Chomczynski and Sacchi (1987). Experimental conditions for RNA electrophoresis, blotting, and hybridization were identical to those we have recently described (Takagi *et al*, 1995). The following cDNA probes were used: a 1.5 kb *Eco*RI fragment of cDNA from the original clone Hf677 for the α1(I) procollagen chain (Chu *et al*, 1982); a 1.4-kb *Pst*I fragment of the cDNA clone pH III 33 coding region for the α 1(III) procollagen chain (Miskulin *et al*, 1986); a 1.1-kb *Eco*RI fragment of the TGF-β1 cDNA (Derynck *et al*, 1985). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was from the American Tissue Culture Collection (ATCC, Rockville, MD). Probes were labeled with ^{32}P by random priming (2×10^8 cpm/μg) and used for Northern blot analysis as previously described (Falanga *et al*, 1993).

Anti-Sense Oligonucleotides We used 0.1 to 10 μM of a TGF-β1 19′mer anti-sense oligonucleotide (5′ gAg ggC ggC ATg ggg gAg g 3′), which overlaps the promoter and transcriptional start site of the TGF-β1 gene. This same sequence, which is specific for the TGF-β1 isoform, has been used successfully by others to block TGF-β1 transcription *in vivo* (Brunet *et al*, 1995). Sense oligonucleotide served as an additional control.

Measurements of Collagenous Protein Cells were seeded in 96-well plates and cultured to a confluent monolayer in 0.2 ml of DMEM supplemented with 10% FBS. After a 48-h incubation to obtain confluent cultures, the monolayers were washed twice with DMEM and incubated 30 min with DMEM supplemented with 0.1% BSA. After washing with DMEM, medium was replaced to 0.2 ml of DMEM, 3% HyClone serum, 50 μg of freshly prepared ascorbic acid per ml, and 2 μCi of [^{3}H]-proline (99 Ci/mmol, Amersham Corp.) with or without TGF-β's. After 24 h, [^{3}H]-proline incorporation into pepsin-resistant salt-precipitable extracellular collagen was determined as previously described (Webster and Harvey, 1979; Takagi *et al*, 1995). Results were expressed as counts per minute (CPM).

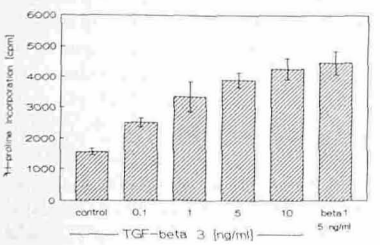

Figure 2. TGF-β3 increases collagenous protein production. [^{3}H]proline incorporation was measured 48 h after the addition of increasing concentrations of TGF-β3 to human dermal fibroblasts. Results represent the mean ± SD from five wells for each experimental group.

Collagen Gel Contraction These were prepared as previously described (Tingstrom *et al*, 1992; Dans and Isseroff, 1994; Takagi *et al*, 1995). Briefly, 35-mm wells of six-well plates were pre-treated overnight with 4 ml of DMEM per well supplemented with 2% BSA to prevent gel attachment to the wells, then washed twice with phosphate-buffered saline. A solution of bovine collagen type I and III (Vitrogen 100, Celtrix Laboratories, Palo Alto, CA) was mixed with 0.2 M HEPES buffer, pH 8.0 (Sigma) and 2 × MCDB 105 (ratio of 4:1:5). After warming the mixture to room temperature, 200 μl of a suspension of 350,000 cells per ml of human neonatal foreskin fibroblasts in MCDB 105 were added per ml, and 2 ml of the final suspension were poured into pre-treated wells and incubated for 1 h at 37°C in 95% air and 5% CO_2. Subsequently, 1 ml of MCDB 105 with or without 15 ng of TGF-β isoforms per ml were added to each gel. As gels were not perfectly round, gel contraction was measured daily by using the means of the major and minor axes as the diameter of the gel. All experiments were carried out in quadruplicate.

Statistical Analysis Data were entered in a computerized statistical analysis program (InStat; GraphPAD Software, San Diego, CA). The Student's t test and one-way analysis of variance test were used for parametric results, whereas linear regression analysis was employed to determine correlation coefficients (r). Statistical significance was defined as a p value of 0.05 or less.

RESULTS

In contrast to the initial hypothesis that TGF-β1 played an important role in the development of neoplasia, it was later found that, for the most part, this and other TGF-β isoforms actually inhibit proliferation of many types of cells, including endothelial and epithelial cells. Fibroblast growth, however, is generally stimulated by TGF-β's (Cox, 1995). In agreement with these earlier observations, we found that TGF-β3 (0.1 to 100 pg per ml) enhanced DNA synthesis in human dermal fibroblast cultures by more than 50% ($p < 0.01$) in a concentration-dependent manner (**Fig 1**; $r = 0.970$). DNA synthesis was maximally stimulated at approximately 100 pg of TGF-β3 per ml, with no further increases observed at higher concentrations (1 to 10 ng per ml; data not shown). We next determined the effect of TGF-β3 on collagenous protein. This was done by measuring [^{3}H]-proline incorporation into pepsin-resistant salt-precipitable extracellular collagen. **Figure 2** shows a representative experiment in which a concentration-dependent ($r = 0.990$), up to 2-fold increase ($p < 0.001$) in collagen synthesis was measured in response to TGF-β3 (0.1 to 10 ng per ml). Maximal increase in collagen synthesis was observed between 1 and 5 ng of TGF-β3 per ml and, as is shown in **Fig 2**, the level of stimulation was similar to that observed with 5 ng of TGF-β1 per ml. Therefore, the above results show that TGF-β3 stimulates both fibroblast DNA synthesis and collagen production. We next tested TGF-β3 in an *in vitro* model of collagen contraction in which fibroblasts contract a collagen gel (Tingstrom *et al*, 1992; Dans and Isseroff, 1994). We prepared collagen lattices (gels) by seeding human dermal fibroblasts in a solution of type I collagen (10). First, we determined the cell number required for optimal gel contraction (data not shown). We then exposed collagen gels to 15 ng of either TGF-β1, β3, or control medium (MCDB 105) per ml. As shown in

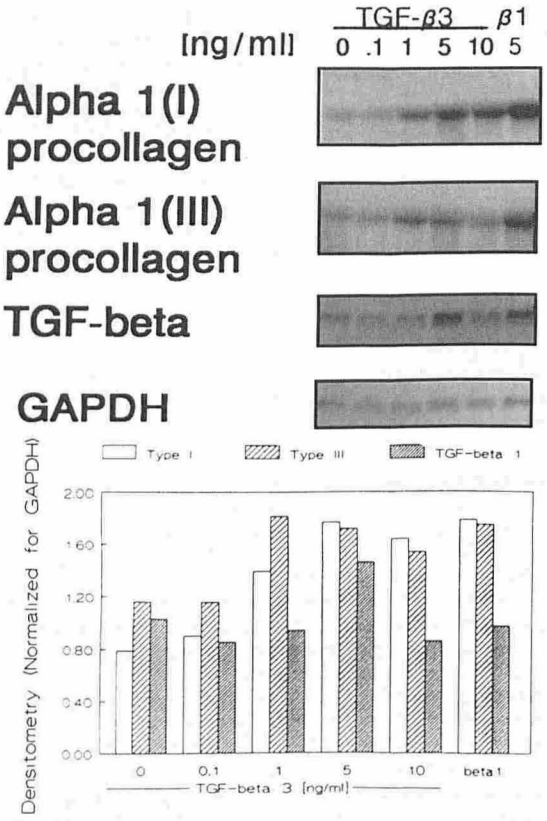

Figure 4. TGF-β3 increases procollagen and TGF-β1 mRNA levels. Human dermal fibroblast cultures were treated with TGF-β3 for 24 h in DMEM plus 10% FBS. The graph shows densitometric readings for α1(I) procollagen, α1(III) procollagen, and TGF-β1, which are normalized for GAPDH.

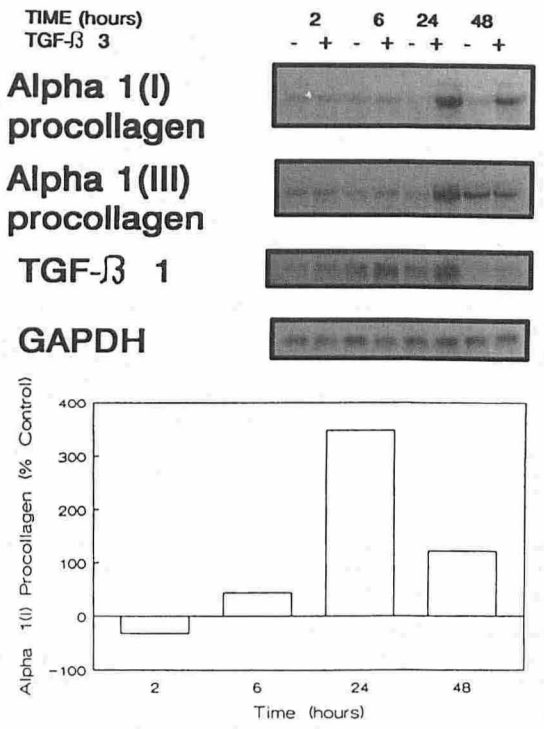

Figure 5. TGF-β3 stimulates procollagen mRNA levels in a time-dependent manner. Human dermal fibroblast cultures were incubated in DMEM plus 10% FBS with or without 5 ng of TGF-β3 per ml. The graph shows densitometric readings for α1(I) procollagen mRNA levels normalized for the expression of GAPDH.

Fig 3, TGF-β3 increased gel contraction as early as 24 h, and by almost 100% ($p < 0.001$) at day 5. This early effect on gel contraction argues against but does not exclude the possibility that the effect may be mediated in part by increased fibroblast proliferation. The gel contraction caused by the two TGF-β isoforms was remarkably comparable in this assay. We next determined whether the stimulation of collagen synthesis by TGF-β3 occurs at the mRNA level. We exposed human dermal fibroblasts to concentrations of TGF-β3 ranging from 0.1 to 10 ng per ml and found that TGF-β3 causes a dose-dependent ($r = 0.990$) more than 2-fold increase in α1(I) procollagen mRNA levels **(Fig 4)**. As with the measurements of collagenous protein synthesis, maximal stimulation of procollagen mRNA was observed between 1 and 5 ng per ml of TGF-β3. In these and in subsequent experiments, it was noted that TGF-β3 leads to the coordinate upregulation of both α1(I) and α1(III) procollagen mRNA. The tight co-regulation of these two collagen genes has been previously described (13). Upregulation by TGF-β3 of mRNA levels of α1(I), α1(III) procollagen, and TGF-β1 was found in five different experiments and was maximal 24 h after the addition of TGF-β3 **(Fig 5)**.

Our results indicate that TGF-β3 increases both procollagen and TGF-β1 mRNA levels. We therefore became interested in knowing the mechanisms by which TGF-β3 stimulates collagen production and whether they are dependent on the action of TGF-β1. We approached this question in two ways. We first used a TGF-β1 anti-sense oligonucleotide, which is specific for the TGF-β1 isoform, to block TGF-β1 transcription in human dermal fibroblast cultures treated with TGF-β3. The 19 mer TGF-β1 anti-sense oligonucleotide used in these experiments has been shown previously to block the action of TGF-β1 *in vivo* (Brunet *et al*, 1995). As shown in **Fig 6**, increasing concentrations (0.1 to 10 μM) of this anti-sense oligonucleotide caused a progressive decrease (more than 50%) in the stimulatory effect of TGF-β3 on α1(I) procollagen mRNA levels ($r = 0.958$). Sense oligonucleotide (10 μM) had no such inhibitory effect and indeed may have caused a small increase in procollagen mRNA levels **(Fig 6)**.

The second approach we used to determine the direct effect of TGF-β3 was to test this isoform in a system devoid of TGF-β1. For this purpose, we cultured fibroblasts from TGF-β1 knock-out mice (Kulkarni *et al*, 1993) and control littermates. As expected, knock-out cells had no mRNA signal for TGF-β1 (not shown). Using these two sets of cells, we found that TGF-β3 is indeed capable of stimulating collagen synthesis on its own, in the absence of the β1 isoform. Thus, when added to dermal fibroblasts derived from TGF-β1 knock-out mice, TGF-β3 was still able to enhance α1(I) procollagen mRNA levels (**Fig 7**; $r = 0.863$). It should be pointed out that in these and in other unrelated experiments we have found that knock-out fibroblasts show a greater baseline synthesis of collagen than their wild-type counterpart. The reasons for this unexpected and paradoxical effect are still unclear to us, but they may represent adjustments or compensatory mechanisms taking place in the absence of TGF-β1 stimulation. Therefore, collagen synthesis is not controlled by TGF-β1 alone, and the effects of other growth factors may be more prominent in TGF-β knock-out cells.

All of the results presented thus far indicate that TGF-β3, like the β1 and β2 isoforms, increases collagen synthesis, α1(I) and α1(III) procollagen mRNA levels, and collagen gel contraction. Our results also show that TGF-β3 increases collagen synthesis through TGF-β1 dependent and independent mechanisms. These observations suggest that TGF-β3 could perhaps control the extent of collagen deposition by shifting from a TGF-β1 dependent to independent pathway. It is unlikely, however, that TGF-β3 acts alone *in vivo*, because release of TGF-β1 by platelets and other cells, including macrophages, is a fundamental event during tissue injury. We therefore hypothesized that the interactions between TGF-β3 with β1 might control the extent of collagen production and asked specifically whether increasing concentrations of TGF-β3 could

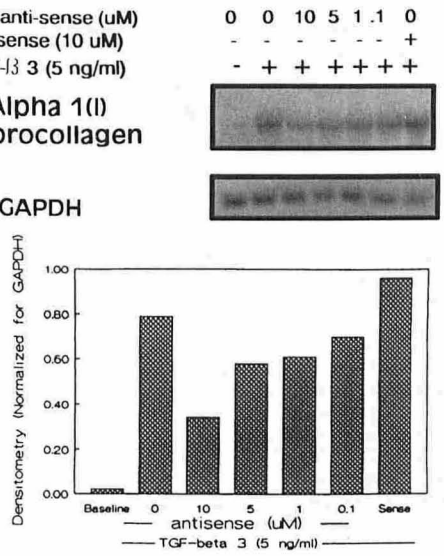

Figure 6. TGF-β1 anti-sense oligonucleotide decreases the stimulatory effect of TGF-β3 on α 1(I) procollagen mRNA. Human dermal fibroblast cultures were first exposed for 24 h to increasing concentrations of a 19 mer TGF-β1 anti-sense oligonucleotide in serum-free medium. Cultures were then either left untreated again or exposed for an additional 24 h to TGF-β3 and to sense oligonucleotide, which served as an additional control. The graph shows densitometric analysis of α1(I) procollagen normalized for GAPDH.

block the stimulatory effects of TGF-β1 on collagen production. **Figure 8** shows first, in agreement with our earlier results, that TGF-β3 increased mRNA for α1(I), α1(III) procollagen, and TGF-β1. Indeed, the combination of small amounts of TGF-β3 (0.1 per ml) with TGF-β1 were at least additive in stimulating procollagen and TGF-β1 mRNA levels. As is shown in **Fig 8**,

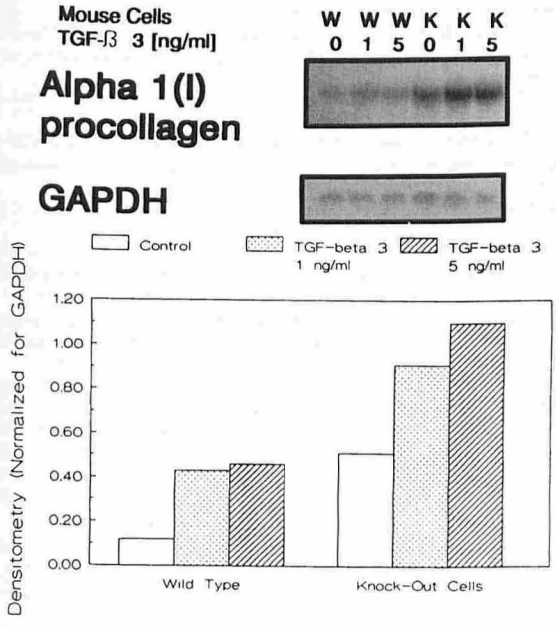

Figure 7. TGF-β3 is capable of stimulating α1(I) procollagen mRNA in TGF-β1 knock-out fibroblasts. Dermal fibroblasts from TGF-β1 knock-out mice (K) and control wild-type (W) littermates were treated overnight with increasing concentrations of TGF-β3 in serum-free medium. The graph represents densitometric readings normalized for GAPDH.

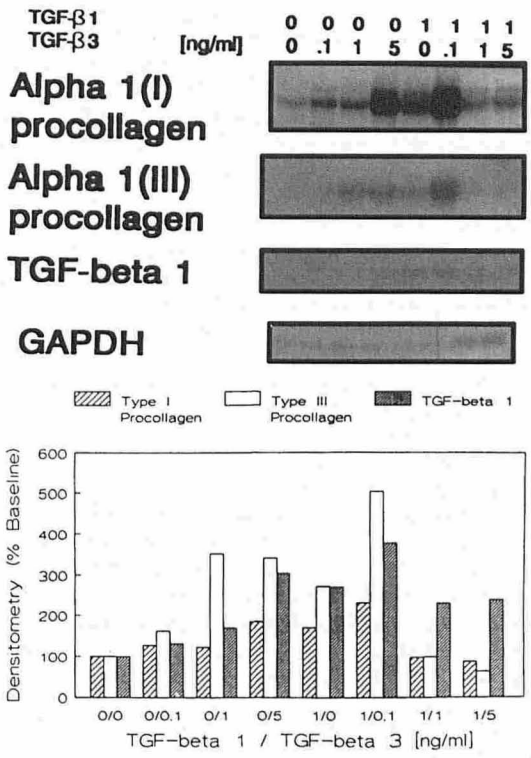

Figure 8. Simultaneous addition of TGF-β3 and TGF-β1 downregulates procollagen mRNA levels. Human dermal fibroblast cultures were incubated for 24 h in serum-free medium after the addition of TGF-β isoforms. The graph represents the densitometric readings for α1(I), αI(III) procollagen, and TGF-β1, which are normalized for the expression of GAPDH. The x axis shows the ratio of different concentrations of TGF-β1and TGF-β3.

however, we found a paradoxical response to the combination of the two isoforms when we increased the concentration of TGF-β3. When added simultaneously with TGF-β1, greater amounts of TGF-β3 (1 and 5 ng per ml) caused a coordinate decrease toward baseline of α1(I), α1(III) procollagen, and TGF-β1 mRNA levels. For this downregulation to occur, the two isoforms must be added in close chronologic sequence. Thus, no decrease in α1(I) or α 1(III) procollagen was observed when the two peptides were added 24 h apart (data not shown).

DISCUSSION

In this report, we show that TGF-β3 stimulates DNA and total collagen synthesis and collagen gel contraction and increases mRNA levels of α1(I), α 1(III) procollagen, and TGF-β1 in cultures of human dermal fibroblasts. Our experiments indicate that TGF-β3 increases collagen synthesis through two pathways, one requiring TGF-β1 transcription and the other independent of this isoform. Moreover, we show that increasing concentrations of TGF-β3 block the stimulatory effects of TGF-β1. These observations indicate that whereas TGF-β3 does increase collagen synthesis and transcription, it may have an important role in controlling the fibrotic effects of TGF-β1 and in fine tuning the synthesis of extracellular matrix within the broad context of stimulation by the TGF-β family of peptides.

Soon after its discovery, TGF-β1 was shown to be an important mediator of extracellular matrix formation and has since been increasingly implicated in the development of fibrosis and scarring (Border and Noble, 1994). It has been repeatedly shown to increase collagen synthesis and transcription (Roberts and Sporn, 1993; Jimenez *et al*, 1994). Because it is stored in platelets and released by other cells, such as macrophages, TGF-β1 is ubiquitously present in relatively large concentrations at sites of tissue injury and of many

inflammatory processes characterized by platelet aggregation and release. Consequently, at least under normal physiologic conditions, certain controls must be in place to prevent or reduce the fibrotic effects of TGF-β1. Activation of the TGF-β1 peptide, modulation of its cellular receptors, and binding of this isoform by matrix molecules and other proteins are some of the established mechanisms involved in controlling the action of TGF-β1 once it is released into the tissues (Roberts and Sporn, 1990, 1993; Cox, 1995; Roberts, 1995). A role for specific TGF-β isoforms in downregulating or controlling the overall responses to TGF-β's is largely unstudied, although autocrine controls are increasingly recognized as important ways to regulate the biologic activity of certain cytokines and growth factors (Sporn and Roberts, 1992). There is direct evidence, however, that TGF-β isoforms are not interchangeable. For example, it was recently shown that inhibition of TGF-β3 (but not TGF-β1 or β2) blocks normal mouse embryo palate fusion (Brunet *et al*, 1995). As another example, rescue from other TGF-β isoforms does not occur in TGF-β1 knock-out mice, which die shortly after birth (Kulkarni, 1993).

The interactions described here between the β1 and β3 isoforms may have important implications for the synthesis of extracellular matrix proteins and for controlling scarring and fibrosis. At least in part, our findings provide an explanation for decreased scarring *in vivo* after the injection of TGF-β3 in experimental *rat* wounds (Shah *et al*, 1995). Although TGF-β3 does stimulate collagen synthesis, it is able to block the stimulatory effects of TGF-β1 on α1(I) and α1(III) procollagen mRNA levels. Interactions between TGF-β3 and TGF-β1 occur in many *in vivo* situations, including wound repair. In addition to being released from platelets, its primary storage site, TGF-β1 is abundant in wounds for other reasons. The proteolytic environment characteristic of wounds can serve to activate the peptide or release it from its stores in extracellular matrix or from the clot formed shortly after wounding (Cox, 1995). Transcription of the TGF-β1 gene in the epidermis and dermis is an early event after injury, and disruption of the basement membrane may provide additional signals for TGF-β1 synthesis (Border and Noble, 1994). While exogenous administration of TGF-β3, as described in previous reports (Shah *et al*, 1995), most probably leads to interactions between this and the TGF-β1 isoform in wounds, there is also experimental evidence that endogenous TGF-β3 is an important player after tissue injury. TGF-β3 is the only isoform that is constitutively expressed in intact human epidermis, and it is detected during wound healing in the migrating epidermis of full-thickness human wounds (Border and Noble, 1994). Therefore, there are many opportunities for TGF-β1 and TGF-β3 to interact *in vivo*, making it possible that the type of control demonstrated here for TGF-β3 could help regulate the overall fibrotic response. At this point we don't know how TGF-β3 enhances collagen synthesis and whether, in its TGF-β1-independent mode, it uses the same mechanisms operative for TGF-β1.

We have shown that TGF-β3 enhances collagen synthesis through TGF-β1–dependent and -independent mechanisms of action and that it can downregulate and limit the action of TGF-β1. This isoform-based and isoform-specific dampening of a biologic response may be a widely operative mechanism for controlling the action of TGF-β's.

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REFERENCES

Border WA, Noble NA: Transforming growth factor β in tissue fibrosis. *N Engl J Med* 331:1286–1292, 1994

Brunet CL, Sharpe PM, Fersugon MWJ: Inhibition of TGF-β3 (but not TGF-β1 or TGF-β2) activity prevents normal mouse embryonic palate fusion. *Int J Dev Biol* 39:345–355, 1995

Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Chem* 162:156–159, 1987

Chu ML, Myers JC, Bernard MP, Ding JF, Ramirez F: Cloning and characterization of five overlapping cDNAs specific for the human proalpha 1 (I) collagen chain. *Nucleic Acids Res* 10:5925–5935, 1982

Cox DA: Transforming growth factor-beta 3. *Cell Biol Int* 19:357–371, 1995

Dans MJ, Isseroff R: Inhibition of collagen lattice contraction by pentoxifylline and interferon-alpha, -beta, and -gamma. *J Invest Dermatol* 102:118–121, 1994

Derynck R, Jarrett JA, Ellson YC, Eaton DH, Bell RJ, Assoian RK, Roberts AB, Sporn MB, Goeddel DV: Human transforming growth factor-beta complementary DNA sequence and expression in normal and transformed cells. *Nature* 316:701–705, 1985

Falanga V, Martin TA, Takagi H, Kirsner RS, Helfman T, Pardes J, Ochoa M: Low oxygen tension increases mRNA levels of alpha 1(I) procollagen in human dermal fibroblasts. *J Cell Physiol* 157:408–412, 1993

Falanga V, Qian SW, Danielpour D, Katz MH, Roberts AB, Sporn MB: Hypoxia upregulates the synthesis of TGF-β1 by human dermal fibroblasts. *J Invest Dermatol* 97:634–637, 1991

Graycar JL, Miller DA, Arrick BA, Lyons RM, Moses HL, Derinck R: Human transforming growth factor-β3: recombinant expression, purification and biological activities in comparison with transforming growth factors-β1 and β2. *Mol Endocrinol* 3:1977–1986, 1989

Jimenez SA, Varga J, Olsen A, Li L, Diaz A, Herhal J, Uitto J: Functional analysis of human α 1(I) procollagen gene promoter. *J Biol Chem* 269:12684–12691, 1994

Kulkarni AB, Hugh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S: Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci USA* 90:770–774, 1993

Miskulin M, Dagleish R, Kluve-Beckermann B, Rennard SI, Tostoshev P, Brantly M, Crystal RG: Human type III collagen gene expression is coordinately modulated with type I collagen genes during fibroblast growth. *Biochemistry* 25:1408–1413, 1986

Roberts AB: Transforming growth factor-β: activity and efficacy in animal models of wound healing. *Wound Rep Reg* 3:408–418, 1995

Roberts AB, Sporn MB: The transforming growth factor-β. In: Sporn MB, Roberts AB (eds.). *Handbook of Experimental Pharmacology. Peptide Growth Factors and Their Receptors*. Springer-Verlag, New York, vol 95:419–472, 1990

Roberts AB, Sporn MB: Physiological actions and clinical applications of tranforming growth factor-β (TGF-β). *Growth Factors* 8:1–9, 1993

Shah M, Foreman DM, Ferguson MWJ: Neutralization of TGF-β1 and TGF-β2 or exogenious addition of TGF-β3 to cutaneous rat wounds reduces scarring. *J Cell Sci* 108:985–1002, 1995

Sporn MB, Roberts AB: Autocrine secretion—10 years later. *Ann Intern Med* 117:408–414, 1992

Takagi H, Ochoa S, Zhou L, Helfman T, Murata H, Falanga V: Enhanced collagen synthesis and transcription by peak E, a contaminant of L-tryptophan preparations associated with the EMS epidemic. *J Clin Invest* 96:2120–2125, 1995

Tingström A, Heldin CH, Rubin K: Regulation of fibroblast-mediated collagen gel contraction by platelet-derived growth factor, interleukin-1α and transforming growth factor-β1. *J Cell Sci* 102:315–322, 1992

Webster DF, Harvey W: A quantitative assay for collagen synthesis in microwell fibroblast cultures. *Anal Biochem* 96:220–224, 1979